Temporary Seronegativity in a Human Immunodeficiency Virus Type 1–Infected Man

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Over a period of 3 months a human immunodeficiency virus 1 (HIV-1)–infected patient showed a sequence of positive-negative-positive anti-HIV screening test results. During this period the level of HIV p24 antigen declined and the HIV antibody pattern by Western blot gradually became complete, suggesting recent HIV infection. However the patient's weight loss, esophageal candidiasis, and *Pneumocystis carinii* pneumonia, together with the severely and persistently lowered CD4 cell counts and the absence of an IgM anti-HIV response, suggest late-stage HIV infection. Despite additional and follow-up testing, it was impossible to determine whether the patient suffered from acute, primary HIV infection with severe immunodepression or from advanced HIV infection (AIDS) with hampered HIV antibody production leading to false-negative test results by the anti-HIV enzyme immunoassay and Western blot. This case illustrates that HIV serology does not always follow the rules. The presence of HIV infection should be considered in a patient showing clinical signs of acute or late-stage HIV infection, even if the anti-HIV assay is negative.
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KEY WORDS: seroconversion; seroreversion; EIA; Western blot; HIV-Ag

INTRODUCTION

The serological events accompanying anti-human immunodeficiency virus (HIV) seroconversion are well known: during a transient peak of HIV-p24 antigenemia the sensitive third-generation anti-HIV enzyme immunoassays (EIAs) become positive, and soon the first anti-HIV reactivity appears by the confirmatory Western blot assay [Busch et al., 1995]. In 50–70% of the cases the patient shows an acute clinical syndrome, generally lasting 1 to 2 weeks [Tindall and Cooper, 1991]. Subsequently, most patients enter an asymptomatic phase of 1 to 10 years before progression to acquired immunodeficiency syndrome (AIDS) occurs. Once seroconversion takes place, anti-HIV EIAs are considered to yield a positive result throughout the remaining life span of the patient. Some HIV-1–infected patients have been described in whom anti-HIV EIAs were persistently negative [U.S. Department of Health and Human Services, 1996; Martin-Rico et al., 1995; Soriano et al., 1994]. We now describe an HIV-1–infected patient showing transient anti-HIV seronegativity.

CLINICAL FINDINGS AND TEST RESULTS

A Dutch patient (male, age 43) visited his physician because of malaise, fever, and weight loss of approximately 15 kg during the last 3 weeks. Physical examination revealed lymphadenopathy in the neck and esophageal candidiasis, which was treated successfully with fluconazole. The local laboratory found a third-generation EIA for HIV antibodies (HIV-1/2 3rd generation plus, Abbott Laboratories, Delkenheim, Germany) to be reactive and submitted a sample for confirmatory testing. Again this HIV-EIA was positive, but the confirmatory Western blot assay (HIV Blot 2.2, Diagnostic Biotechnology, Singapore) was negative. To avoid missing very early stages of HIV infection, HIV-antigen (HIV-Ag) testing should be undertaken in such cases [Zaaijer et al., 1992]. The HIV-Ag assay (Abbott Laboratories, Chicago) tested maximum positive and was confirmed by neutralization.

To confirm further HIV infection follow-up samples were studied (see Fig. 1). Surprisingly, the second and third sample, respectively, showed borderline and negative test results by anti-HIV EIA. The HIV antibody reactivity gradually re-appeared in subsequent samples. In the Western blot this phenomenon was not observed: the antibody pattern gradually increased, starting with the second sample. In the sixth sample, the HIV-1 Western blot pattern was complete, and a Western blot for HIV-2 antibodies was negative (Diag-

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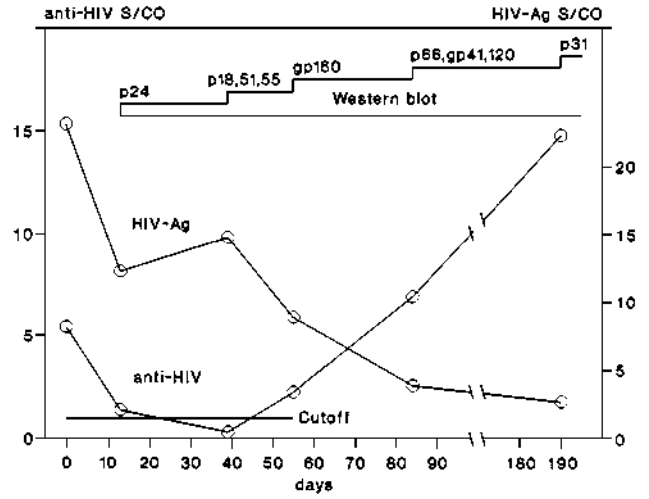

Fig. 1. HIV serology in a case of temporary seroreversion. S/CO = EIA sample to cut-off ratio, positive if ≥1.

nostic Biotechnology, Singapore). Technical errors and a mix-up of samples can be excluded as the anti-HIV EIAs and Western blots were performed using the original sample tubes and identical test results were obtained in repeat test runs by different technicians. The HIV-Ag assay showed neutralizable positive test results of decreasing strength in the follow-up samples. Testing the fourth sample, the polymerase chain reaction (PCR) for HIV-1 DNA (Roche) was positive, and quantitative determination of HIV-1 RNA (Organon) was strongly positive: 1,500,000 copies/ml. Zone electropheresis showed elevated levels of polyclonal IgG with oligoclonal IgGs, as observed in patients suffering from multiple infections. The lymphocyte count, the CD4 count, and the CD4/CD8 ratio were severely lowered (see Table I). Contrary to previous observations in several seroconversions [Roos et al., 1992], CD8+ lymphocytosis did not occur. In experimental immunoblots, the antibody response against synthetic HIV-envelope peptides was typical for European and American HIV-1 isolates; no significant reactivity occurred against peptides from HIV-O and other African isolates (immunoblotting kindly performed by Innogenetics, Antwerp, Belgium). An experimental EIA showed borderline levels of IgM antibodies to HIV-1 (IgM EIA kindly performed by Abbott Laboratories, Delkenheim).

The follow-up samples 2–5 could be tested by four alternative anti-HIV EIAs (see Table I): two "double antigen sandwich assays" (from Organon Teknika and Murex Diagnostics) and two "anti-antibody assays" (from Abbott Laboratories and Ortho Diagnostic Systems). The Organon assay tested repeatedly barely below the positive/negative threshold value on the second and third sample, which originally tested positive resp. negative in the Abbott third-generation-plus EIA. Both "anti-antibody" EIAs only turned positive on the fifth sample, 84 days after the patient originally was found positive in the third-generation-plus EIA.

On day 84 the patient was feverish, suffered from dyspnea, and coughed. *Pneumocystic carinii* pneumonia (PCP) was diagnosed by bronchoalveolar lavage, and responded to cotrimoxazole therapy. The patient denies HIV-related risk factors.

DISCUSSION

Our patient showed a puzzling set of test results. A widely used HIV screening test gradually changed from positive to negative and back to positive again, but at the same time the Western blot pattern steadily grew complete. Thirty-nine days after the HIV antigen first was detected five different HIV antibody assays tested negative. Two anti-HIV EIAs were found negative until PCP was diagnosed on day 84. It remains unclear whether the patient suffered from acute, primary HIV infection or from late-stage HIV infection (AIDS). The

TABLE I. HIV Serology and Cell Counts

	Day 0	Day 13	Day 39	Day 55	Day 84	Day 189
HIV serology						
Anti-HIV (Abbott 3.0+ EIA)	5.41	1.37	0.32	2.26	6.91	>14.81
HIV antigen (Abbott)	>23.0	12.21	14.75	8.82	3.86	2.70
IgM anti-HIV (Abbott)	.	1.12	1.44	1.36	1.41	.
Western blot (DB) pattern						
Gag	Neg	24	18,24,55	18,24,55	18,24,55	18,24,55
Pol	Neg	Neg	51	51	51,66	31,51,66
Env	Neg	Neg	Neg	160	41,120,160	41,120,160
Additional HIV-EIAs						
Organon Uniform II	.	0.98	0.98	3.50	4.43	.
Murex 3rd gen.	.	0.51	0.35	1.97	6.94	.
Abbott 2nd gen.	.	0.24	0.26	0.71	3.78	.
Ortho HIV-1/2	.	0.07	0.07	0.90	8.31	.
Cell counts (normal range)						
Lymphocyts (1,260–3,270)	410	.	.	.	300	800
CD4+ cells (560–1,490)	110	.	.	.	50	230
CD8+ cells (260–990)	220	.	.	.	180	340
B-cells (>60)	30	.	.	.	20	120
CD4/CD8 ratio (1.01–3.99)	0.50	.	.	.	0.28	0.68

• = not tested. All EIA results: sample/cut-off ratios, positive if ≥1.0. All cell counts $\times 10^6$/L.

white cell counts, the severe weight loss, the *Pneumocystis carinii* pneumonia, the zone electrophoresis pattern, and the absence of an IgM anti-HIV response suggest late-stage HIV infection. However, to explain a blank Western blot during late-stage HIV infection one must assume a complete and prolonged stop of HIV antibody production in the preceding months, or a total complexation of all HIV antibodies by HIV-antigens, which both are hard to imagine.

The gradual appearance of bands in the Western blot, the waning HIV-Ag signal, and the partial recovery of the cell counts suggest recent HIV infection. Esophageal candidiasis during acute primary HIV infection has been observed by us and by others [Mientjes et al., 1993]. Possibly the patient experienced recent HIV infection with an unusually aggressive course, involving severe immunosuppression that caused hampered HIV antibody production and opportunistic infections. Another explanation would be the presence of an unknown factor during seroconversion causing transient false-negative EIA test results, but not interfering with Western blotting. It seems unlikely that during seroconversion complexation of antibodies by HIV-antigens temporarily blocked antibody detection because the HIV-Ag level was already declining at the moment of seronegativity.

This case illustrates the value of HIV-Ag testing on clinical samples: The HIV-Ag assay confirmed HIV infection both in an HIV-EIA–reactive/Western blot–negative sample and in an HIV-EIA–negative/Western blot–reactive sample. Recently the persistent failure to detect seroconversion in an HIV-1 infected man was reported [U.S. Department of Health and Human Services, 1996]. Our patient showed transient seroreversion. We conclude that HIV serology does not always follow the rules. The presence of HIV infection should be considered in a patient showing clinical signs of acute or late-stage HIV infection, even if the anti-HIV assay yields negative results.

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